## Gene Isoform Specificity through Enhancer-Associated Antisense Transcription.

**Journal:** PLoS One

**Publication Year:** 2012

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**PubMed link:** 22937057

**Funding Grants:** Training Program in Systems Biology of Stem Cells

**Public Summary:**
Regulation of gene expression is critical in cell differentiation and in establishing cell identity. We report a novel mechanism of gene regulation discovered in neural differentiation of mouse embryonic stem cells (mESCs). This novel mechanism involves enhancers and antisense ncRNAs, both of which have been historically known to regulate gene expression in different and independent ways. Using RNA-Sequencing to probe mESCs and their derived neural precursor cells (NPs), we identified two novel enhancer-associated antisense ncRNAs that appear to control isoform-specific expression of their overlapping protein-coding genes. In each case, an enhancer internal to a protein-coding gene drives an antisense ncRNA in mESCs but not in NPs. Expression of the antisense ncRNA is correlated with expression of a shorter isoform of the associated sense gene that is not present when the antisense ncRNA is absent. We demonstrate that expression of the antisense ncRNAs as well as expression of the short sense isoforms correlates with enhancer activity at these two loci. Further, overexpression and knockdown experiments suggest the antisense ncRNAs regulate expression of their associated sense genes via cis-acting mechanisms. Interestingly, the protein-coding genes involved in these two examples, Zmynd8 and Brd1, share many functional domains, yet their antisense ncRNAs show no homology to each other and are not present in non-murine mammalian lineages, such as the primate lineage. The lack of homology in the antisense ncRNAs indicates they have evolved independently of each other and suggests that this mode of lineage-specific transcriptional regulation may be more widespread in other cell types and organisms. Our findings present a new view of enhancer action wherein enhancers may direct isoform-specific expression of genes through ncRNA intermediates.

**Scientific Abstract:**
Enhancers and antisense RNAs play key roles in transcriptional regulation through differing mechanisms. Recent studies have demonstrated that enhancers are often associated with non-coding RNAs (ncRNAs), yet the functional role of these enhancer:ncRNA associations is unclear. Using RNA-Sequencing to interrogate the transcriptomes of undifferentiated mouse embryonic stem cells (mESCs) and their derived neural precursor cells (NPs), we identified two novel enhancer-associated antisense transcripts that appear to control isoform-specific expression of their overlapping protein-coding genes. In each case, an enhancer internal to a protein-coding gene drives an antisense RNA in mESCs but not in NPs. Expression of the antisense RNA is correlated with expression of a shorter isoform of the associated sense gene that is not present when the antisense RNA is not expressed. We demonstrate that expression of the antisense transcripts as well as expression of the short sense isoforms correlates with enhancer activity at these two loci. Further, overexpression and knockdown experiments suggest the antisense transcripts regulate expression of their associated sense genes via cis-acting mechanisms. Interestingly, the protein-coding genes involved in these two examples, Zmynd8 and Brd1, share many functional domains, yet their antisense ncRNAs show no homology to each other and are not present in non-murine mammalian lineages, such as the primate lineage. The lack of homology in the antisense ncRNAs indicates they have evolved independently of each other and suggests that this mode of lineage-specific transcriptional regulation may be more widespread in other cell types and organisms. Our findings present a new view of enhancer action wherein enhancers may direct isoform-specific expression of genes through ncRNA intermediates.